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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/175,683	10/20/1998	LI HOW CHEN	10275-134001	7184

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EXAMINER	
SCHNIZER, RICHARD A	

ART UNIT	PAPER NUMBER
1635	35

DATE MAILED: 03/10/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Advisory Action

Application No.	Applicant(s)
09/175,683	CHEN
Examiner	**Art Unit**
Richard Schnizer	1635

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED *Feb 19, 2003* FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE. Therefore, further action by the applicant is required to avoid the abandonment of this application. A proper reply to a final rejection under 37 CFR 1.113 may only be either: (1) a timely filed amendment which places the application in condition for allowance; (2) a timely filed Notice of Appeal (with appeal fee); or (3) a timely filed Request for Continued Examination (RCE) in compliance with 37 CFR 1.114.

THE PERIOD FOR REPLY [check only a) or b)]

a) ☐ The period for reply expires _____ months from the mailing date of the final rejection.

b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection. ONLY CHECK THIS BOX WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

1. ☒ A Notice of Appeal was filed on *Feb 19, 2003*. Appellant's Brief must be filed within the period set forth in 37 CFR 1.192(a), or any extension thereof (37 CFR 1.191(d)), to avoid dismissal of the appeal.

2. ☒ The proposed amendment(s) will not be entered because:

(a) ☒ they raise new issues that would require further consideration and/or search (see NOTE below);

(b) ☐ they raise the issue of new matter (see NOTE below);

(c) ☐ they are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or

(d) ☐ they present additional claims without canceling a corresponding number of finally rejected claims.

NOTE: *The new claims raise a variety of issues not previously searched or considered, e.g. claim 49 is drawn to a protein.*

3. ☐ Applicant's reply has overcome the following rejection(s): _____

4. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).

5. ☒ The a) ☐ affidavit, b) ☐ exhibit, or c) ☒ request for reconsideration has been considered but does NOT place the application in condition for allowance because:
See attached.

6. ☐ The affidavit or exhibit will NOT be considered because it is not directed SOLELY to issues which were newly raised by the Examiner in the final rejection.

7. ☒ For purposes of Appeal, the proposed amendment(s) a) ☒ will not be entered or b) ☐ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.

The status of the claim(s) is (or will be) as follows:

Claim(s) allowed: *None*

Claim(s) objected to: _____

Claim(s) rejected: *6-8, 10, 20, 31-37, and 48*

Claim(s) withdrawn from consideration: _____

8. ☐ The proposed drawing correction filed on _____ is a) ☐ approved or b) ☐ disapproved by the Examiner.

9. ☐ Note the attached Information Disclosure Statement(s) (PTO-1449) Paper No(s). _____.

10. ☐ Other:

ADVISORY ACTION

Had they been entered, Applicant's amendments would have overcome the new matter and indefiniteness rejections of claims 6-8, 10, 20, 31-37, and 48.

1. *Applicant's request for reconsideration has been considered, but does not place the application in condition for allowance.*

Claims 35-37 stand rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods of making polypeptides encoded by SEQ ID NO:1, polypeptides identical to those encoded by SEQ ID NO:1 except that they comprise N182Q and N263Q mutations, and fragments of these polypeptides, by providing a non-human transgenic mammal whose genome comprises a nucleic acid encoding the polypeptide or polypeptide fragment, wherein the nucleic acid has been modified by codon optimization, or removal of AUUUA motifs, or both, wherein the nucleic acid is operably linked to a transcription control sequence which causes transcription in a mammary gland of the animal, and wherein the animal secretes the polypeptide or polypeptide fragment into milk of the animal in an amount that is at least 25%, 50% or 100%, greater than the amount observed under the same conditions for animals comprising unmodified nucleic acids encoding these polypeptides or fragments, does not reasonably provide enablement for methods of producing these amounts of any other parasite protein in a transgenic animal. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The invention is a method of making any parasite protein in the milk of a transgenic mammal.

The essence of the invention is the discovery by Applicant that the Plasmodium falciparum MSP-1 protein is not expressed in cultured mammalian cells or in transgenic mice if the gene encoding MSP-1 is not modified from its native state. Applicant has shown that specific sequence modifications which increase the GC-content of an MSP-1 transgene, and which remove the AUUUA motifs from the gene, result in expression of the encoded protein in cultured mammalian cells and in the milk of transgenic mice.

The prior art teaches that codon optimization and/or removal of AUUUA motifs can lead to improved expression of heterologous proteins in mammalian expression systems. See Dziegiel, Seed, and Bosch below under 35 USC 103 rejections. However, the prior art also teaches that the amount to which protein expression can be improved by such modifications is unpredictable. It is assumed in the art that the use of codons corresponding to abundant charged tRNAs results in faster translation and fewer stalled ribosomes. This makes mRNAs poorer targets for RNases, thereby stabilizing them. See Seed (1998) column 1, lines 15-24. It is also known that AUUUA motifs can destabilize mRNAs depending on the presence in the cell of the appropriate destabilizing proteins which bind to these sequences. AUUUA motifs have been shown to be active when located either in the coding region or the 3'-untranslated regions of transcripts. See Akashi et al (1994), abstract and Fig. 1. However, as Applicant points out in Paper No. 16, Akashi also teaches that there are other factors unrelated to AUUUA sequences which play a role

in mRNA destabilization. In support of this Liebhaber (1997) teaches that primary, secondary, and tertiary structures of mRNAs influence their stability by determining their exposure to nucleases, However, Liebhaber also notes that the specific higher order structural determinants of mRNA stability were unknown at the time the invention was filed. See abstract.

Applicant has shown that the invention can be used to successfully increase the expression of MSP-1 protein in mammalian cells. Evidence is disclosed which strongly suggests that the invention functions to stabilize MSP-1 mRNA. For this reason, the invention should be useful in increasing the expression of proteins in situations where the primary hindrance to expression is unstable mRNA, assuming that the instability of the mRNA owes to the presence of AUUUA motifs or degradation due to slow translation. However, as discussed above, Akashi and Liebhaber teach that the factors controlling mRNA stability are not limited to the presence of AUUUA motifs and the rate of translation, and can involve secondary and tertiary structures of the message. Because the higher order structural determinants which affect mRNA stability have not been determined and remain under study, one cannot predict a priori how much a given sequence change will improve the stability of a given mRNA, and how much of an improvement in protein production will result. Rather one must examine each case individually, and determine empirically what are the effects of each mutation. One might argue that it is not undue experimentation to determine the degree of stabilization which can be conferred by each particular sequence modification. However as set forth in *In Re Fisher*, 166 USPQ 18(CCPA 1970), compliance with 35 USC 112, first paragraph requires:

> that the scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to **known scientific laws**; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with the degree of unpredictability of the factors involved.

Emphasis added. Because the nature and identity of mRNA destabilizing sequences is highly unpredictable, and the specification fails to provide any means to predict which sequences will destabilize mRNAs, and by what amount, one of skill in the art would have to perform undue experimentation in order to obtain predictable amounts of increased protein expression by use of the claimed methods.

Response to Arguments

2. Applicant's arguments filed 2/19/03 have been fully considered but they are not persuasive.

Applicant considers the enablement rejection at pages 13-15 of the response. The argument is unpersuasive because Applicant fails to consider the level of unpredictability in the art, which is the basis of the enablement rejection. Applicant's argument is based on the fact that the specification gives a single working example of the invention. The rejection clearly states that the specification is enabling for the scope of this example, however the problem arises in extending the scope of the invention to the expression of other proteins. The claims require discrete improvements in the level of expression of proteins, i.e. 25, 50 or 75% greater expression than that observed in the absence of the claimed DNA sequence modifications. As set forth above

in the rejection, there are many unknown factors governing the quantity of expression of polypeptides, and this art is highly unpredictable. Applicant has failed to address this aspect of the rejection, so the rejection is maintained.

3. Claims 6-8, 10, 20, 31-34, and 48 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Dziegiel (1993), Seed (1998), Akashi (1994), Bosch (1994), and Black (1996).

The invention is a method of making any parasite protein in the milk of a transgenic mammal. The invention requires an optimized transgene designed to be expressed at a high level in mammary tissue. Optimization includes selection of codons known to occur in highly-expressed milk proteins, and the removal of AUUUA mRNA-destabilization motifs from the transgene.

Dziegiel teaches an expression vector comprising a nucleic acid encoding an antigen of *Plasmodium falciparum*. See abstract. The expression vector may be used in mammalian cells for the purpose of producing and isolating the antigen, and may be used to construct transgenic animals for the purpose of producing the antigen. The polypeptide produced may be used as a vaccine. See column 18, lines 54-65, and column 19, lines 61-63. The nucleic acid may be modified by silent mutations which favor the codon usage of the organism in which the nucleic acid will be expressed. See column 20, line 66 to column 21, line 7; and column 21, lines 36-40. The nucleic acid encoding the antigen is only 30% G+C, and comprises at least two AUUUA

motifs within the coding region. See column 16, lines 40-43, and bases 962-966, and 1896-1900 in the sequence bridging columns 13 and 14.

Dziegiel does not specifically recommend reducing the AT-content of the nucleic acid, the removal of mRNA instability motifs, or the production of the antigen in milk of the transgenic animal.

Seed teaches that codon optimization may be used to increase the expression of foreign genes in mammalian cells. See column 1, lines 8-10; and column 2, lines 7-11. Preferred codons are always those with the highest possible GC-content. See lines 33-37, and Table 1, bridging columns 7 and 8. For amino acids A, R, N, Q, H, I, L, K, P, F, and S, Seed teaches that the most preferred codon is the same codon which Applicant chose to use most frequently in reducing the invention to practice. Compare Fig. 3A of the instant application to Table 1 of Seed. Seed also teaches avoiding the use of AUUUA motifs in synthetic genes. See column 12, lines 35-37.

Akashi teaches that the function of AUUUA motifs is not restricted to their location within the mRNA. These motifs need not be located in the 3'-untranslated region of mRNAs, and are capable of destabilizing mRNAs even when located in the coding region. See abstract, and Fig. 1

Bosch teaches removal of mRNA instability motifs from nucleic acids which are intended to be expressed in heterologous hosts. Bosch also teaches that codon optimization is advisable. See column 4, lines 12-21.

Black teaches a method of expressing recombinant proteins in the milk of female mammals. The method comprises operatively linking an alpha-ovalbumin promoter to an exogenous gene sequence and generating a transgenic animal comprising the hybrid gene. The alpha-lactalbumin promoter directs expression in mammary tissue. The expressed protein is secreted into the mammal's milk, from which it can be purified. See column 2, lines 34-46. The transgenic animals may comprise the construct in their germ line cells. See column 9, line 49 to column 10, line 31, especially column 10, lines 29-31.

It would have been obvious to one of ordinary skill in the art at the time of the invention to optimize the codon usage of the transgene of Dziegiel as taught by Seed. One would have been motivated to do so because Seed teaches that codon optimization can improve expression of foreign genes in mammalian cells. One would have been motivated to decrease the AT-content of the transgene because it is apparent from the teachings of Seed that the most preferred codons in mammalian systems are the most GC-rich codons. Similarly one would have been motivated to remove AUUUA motifs from the transgene because Seed teaches that this should be done. Furthermore, it would be inherent in the process of selecting GC-rich codons. For example, depending on the reading frame, the sequence AUUUA can comprise an AUU codon encoding I, a UUU codon encoding F, or a UUA codon encoding L. The preferred codon for each of these amino acids, as taught by Seed, comprises a G or C. Thus if one followed the teachings of Seed in terms of codon selection, one would necessarily remove AUUUA motifs from the transgene open reading frame. One also would have been motivated to remove AUUUA sequences from

the transgene open reading frame because Akashi teaches that AUUUA sequences in open reading frames can destabilize mRNA. One would have been motivated to produce the parasite protein in the milk of the transgenic animal because Black teaches that transgenic recombinant polypeptides can be produced in the milk of transgenic animals, and that the produced proteins can be non-native to the animal, and can be used industrially. See column 4, lines 51-57. The obviousness of optimizing codon usage and removing AUUUA sequences from genes intended to be expressed in heterologous organisms is underlined by Bosch who suggests both of these practices.

Thus the invention as a whole was prima facie obvious.

Response to Arguments

4. Applicant's arguments filed 2/19/03 have been fully considered as they apply to the rejection above but they are not persuasive.

Applicant considers the rejection at pages 16-29 of the response.

At pages 17-20, Applicant argues that Dziegiel is not an appropriate reference and does not fall within the scope of the applicable prior art. This is unpersuasive because Dziegiel teaches that *Plasmodium falciparum* antigens should be expressed in heterologous hosts, including transgenic animals, and that the codons of the transgene should be optimized for maximum expression in each host. Clearly, Dziegiel is related to the same field of endeavor as the claimed invention, and is pertinent to the problem of protein expression inasmuch as codon optimization is suggested for the purpose of improving expression of genes in heterologous hosts. Thus, Dziegiel

satisfies the criteria for analogous art set forth by Applicant at page 19 of the response, and Applicant's contention at page 20 of the response that the teachings of Dziegiel have "nothing to do with the myriad of expression problems overcome by the instant claims" and "are not pertinent to the claimed invention" is clearly incorrect. It is true that Dziegiel does not teach each and every aspect of the claimed invention. However, Applicant is reminded that if Dziegiel taught each and every limitation of the invention, the rejection would have been made under 35 USC 102, not 35 USC 103. Because the rejection is made under 35 USC 103, other references are relied upon to provide what is missing from Dziegiel.

At pages 20-22 Applicant argues that Dziegiel teaches away from the claimed invention. This is clearly incorrect for the reasons given above, i.e. because Dziegiel teaches that *Plasmodium falciparum* antigens should be expressed in heterologous hosts, including transgenic animals, and that the codons of the transgene should be optimized for maximum expression in each host. In order to teach away from the instant invention, Dziegiel would have had to indicate that it was not possible or feasible to obtain expression of *Plasmodium falciparum* antigens in transgenic animals. In fact, Dziegiel teaches the opposite, and suggests that the transgene sequence should be altered for best expression in the host of choice. Applicant's argument at pages 21 and 22 that the invention of Dziegiel is inoperable is unsupported. Applicant offers no reason as to why one would not be able to obtain expression of any transgene in a transgenic animal if the codons of the transgene are suitably optimized for that animal.

Applicant's opinion expressed at page 23 of the response that the invention addresses a long felt need in the art for a reliable parasite protein expression system is noted. However, it is not considered sufficient to overcome the obviousness rejection because Applicant has failed to explain exactly why it renders the claims non-obvious over the prior art.

Applicant considers the prima facie case of obviousness at pages 24-28 of the response.

At page 24 Applicant asserts that Dziegiel fails to provide motivation to combine with other prior art. This is unpersuasive because Dziegiel explicitly suggests that transgene codons should be optimized for the system in which the transgene should be expressed. Thus one seeking to express a transgene in a transgenic animal, as suggested by Dziegiel, would look to the art to determine the how best to optimize codons. On looking the art, one would find Seed who not only teaches that GC content should be maximized, but also teaches that AUUUA motifs should be avoided. See column 12, lines 35-37.

At pages 25-29 Applicant considers the other citations relied on in the rejection. Applicant is reminded that one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Applicant has failed to show that the references do not teach each and every element of the claims, or that one of ordinary skill in the art would lack the motivation to combine them to arrive at the instant invention.

From the cited references it is clear that the expression of parasite antigens in transgenic animals had been considered in the prior art. Problems associated with the expression of proteins in heterologous systems, including transgenic animals, had been considered and codon optimization and removal of AUUUA motifs were solutions known to those of ordinary skill in the art at the time of the invention. Finally, expression of foreign proteins in the milk of transgenic animals was well known in the prior art as a means of conveniently obtaining such proteins. For all these reasons the rejection is maintained.

Any inquiry concerning this communication or earlier communications from the examiner(s) should be directed to Richard Schnizer, whose telephone number is 703-306-5441. The examiner can normally be reached Monday through Friday between the hours of 6:20 AM and 3:50 PM. The examiner is off on alternate Fridays, but is sometimes in the office anyway.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, John Leguyader, can be reached at 703-308-0447. The FAX numbers for art unit 1632 are 703-308-4242, and 703-305-3014. Additionally correspondence can be transmitted to the following RIGHTFAX numbers: 703-872-9306 for correspondence before final rejection, and 703-872-9307 for correspondence after final rejection.

Inquiries of a general nature or relating to the status of the application should be directed to the Patent Analyst Trina Turner whose telephone number is 703-305-3413.

Richard Schnizer, Ph.D.

JEFFREY SIEW
PRIMARY EXAMINER
2/28/03